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FoxOs in Tumor Suppression and Stem Cell Maintenance

Karen C. Arden[1,*]
[1]Ludwig Institute for Cancer Research and Department of Medicine, University of California, San Diego, School of Medicine, La Jolla, CA 92093, USA
*Correspondence: karden@ucsd.edu
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The FoxO transcription factors have been implicated in many processes including tumor suppression and cell death. In this issue, two groups now report on mice that conditionally lack the three predominant FoxO transcription factors. Tothova et al. (2007) demonstrate that FoxOs are critical for the long-term maintenance of hematopoietic stem cells, and Paik et al. (2007) show that FoxOs suppress the formation of hemangiomas and lymphomas in mice.

The three principal members of the mammalian FoxO subfamily of Fox transcription factors (FoxO1, FoxO3, and FoxO4) were first discovered through the cloning of chromosomal translocation breakpoints associated with tumors (reviewed in Arden, 2006). FoxOs have since been studied through manipulation of cultured cells and in worms, flies, and mice (reviewed in Greer and Brunet, 2005). They are important in a variety of processes including cellular differentiation, tumor suppression, metabolism, cell-cycle arrest, cell death, and protection from stress. In mice, the individual disruption of each of the three FoxO genes results in different phenotypes, suggesting some degree of functional diversification during development (Castrillon et al., 2003, Hosaka et al., 2004). Yet the three genes have overlapping patterns of expression and all three bind to the same DNA target sequence. Thus, the FoxOs may be capable of regulating the same target genes and so may have overlapping functions. To address this possibility, two groups now report in this issue the consequences of the conditional and simultaneous disruption of both alleles of the three principal FoxO genes in mice (Paik et al., 2007; Tothova et al. 2007).

Paik et al. (2007) describe the phenotype of animals in which Cre-mediated gene disruption of all three *FoxO* genes was induced at 4 to 5 weeks of age. Between 19 and 30 weeks of age the animals developed lymphoblastic thymic lymphomas that were capable of spreading to the spleen, liver, and lymph nodes (Table 1). In addition, as early as 6 to 8 weeks of age, mice also developed widespread hemangiomas starting in the uterus that progress to massive fatal hemangiomas affecting numerous tissues. In 9% of the animals the lesions progressed to lethal angiosarcomas. Loss of all three *FoxO* alleles is required for the full tumor phenotype, as disruption of any two of the three *FoxO* genes resulted in a less severe phonotype. This confirms that there is functional redundancy among these closely related transcription factors.

Given the widespread tissue distribution of FoxO expression in mammals it is somewhat surprising that the tumor phenotype of these mice is restricted to only certain tissues. The chromosome translocations that disrupt the human *FOXO* gene have been associated with leukemia and the solid tumor alveolar rhabdomyosarcoma, and misregulation of FOXOs has been associated with a variety of tumor types, including those of the prostate, stomach, brain, and breast. Because the FOXO DNA-binding domain is disrupted by the chromosomal translocations it has been proposed that FoxOs may function as tumor suppressors. As would be expected of a tumor-suppressor gene, activation of FoxO factors by mutation of the phosphorylation sites (thereby restricting the localization of the FOXO proteins to the nucleus) leads to cell-cycle arrest or cell death. Furthermore, all three FoxO factors are directly regulated by AKT, and the tumor spectrum resulting from mutations affecting the PI3K/AKT pathway produce a variety of tumor types. Thus, it is surprising that, despite the absence of FoxO in several cell and tissue

Table 1. Phenotypes of the *FoxO1*, *FoxO3*, *FoxO4* Conditional Knockout Mouse

Tissue	Phenotype
Bone Marrow	Decrease in long-term hematopoietic stem cell population due to increased entry into cell cycle, decreased renewal capacity, increased apoptosis, and an increase in reactive oxygen species.
Vasculature	Uterine hemangiomas appear at 6 to 8 weeks of age, followed by widespread fatal hemangiomas in skeletal muscle, abdominal wall, liver, adrenal glands, bone marrow, lymph nodes, and skin.
Thymus	Thymic lymphomas appear at 19 to 30 weeks of age; they may subsequently spread to spleen, liver, and lymph nodes.

types where FoxOs are normally expressed, the tumor phenotype was restricted to thymocytes and endothelial-derived cells.

Remarkably, not all tissues containing endothelial cells developed hemangiomas. For example, lungs and kidneys remained unaffected, suggesting that the phenotype is not only restricted to particular cell lineages but is also organ specific. A closer look at the differences between cell types revealed that FoxO-deficient endothelial cells derived from the liver, like FoxO-deficient thymocytes, have an increased ability to proliferate and survive. These aberrant characteristics were absent in FoxO-deficient endothelial cells derived from lung tissue. When these two cell types were subjected to gene expression profile studies, 138 genes were identified as differentially expressed. Genes likely to be direct FoxO targets were predicted using an in silico approach to identify known FoxO-binding motifs. This process narrowed the list to 21 genes, 12 of which could be validated by more than one method. The most significant change in gene expression found in the FoxO-deficient endothelial cells of the liver compared to those of the lung is the downregulation of *Sprouty2*, a protein known to inhibit growth factor signaling. The authors establish *Sprouty2* as a negative regulator of endothelial cell proliferation and survival and a major effector of FoxO function in the endothelium. Interestingly, the same in silico approach, when applied to FoxO-deficient thymocytes (the thymus being the other tissue from which tumors originated), produced a nonoverlapping list of putative direct targets further reinforcing the concept of FoxO cell and tissue type specificity. Although much remains to be elucidated concerning the role of FoxOs in tumorigenesis, this report lays an invaluable groundwork for future studies.

The work by Tothova et al. (2007) implicates FoxOs as important mediators of the cellular response to oxidative stress, which is involved in the etiology of many human diseases. Using the same system as Paik et al. to disrupt all three FoxO genes, Tothova et al. focused on the hematopoietic system. They detected a significant decrease in the population of long-term hematopoietic stem cells (HSCs) and in the common lymphoid progenitor compartment. The FoxO-deficient bone marrow was defective in colony formation activity in vitro and in its ability to support long-term bone marrow repopulation in vivo while short-term repopulation activity was slightly increased. The authors also found a significant increase in the number of HSCs, but not myeloid progenitors, exiting G0/G1 and entering S/G2/M, suggesting that FoxOs are important in maintaining HSCs in the quiescent state, thereby preserving their replicative and self-renewal capacity. The FoxO-deficient HSCs also display an increased level of apoptosis further contributing to the aberrant decrease in cell number. Unlike the cell-cycle phenotype, the increase in apoptosis was not restricted to HSCs but was also detected in more mature populations as well. Corroborating the functional redundancy of FoxO proteins observed by Paik et al., deficiency in any one or two FoxO genes in combination does not produce the full cell cycle and apoptotic phenotype. FOXO4 had previously been shown to play a role in the resistance of quiescent cells to reactive oxygen species (Kops et al., 2002). Tothova et al. found a marked increase in the levels of reactive oxygen species in HSCs isolated from the FoxO-deficient mice. Gene expression analysis, focusing on genes involved in the metabolism of reactive oxygen species, revealed a decrease in expression of a subset of genes associated with the cellular response to oxidative stress. This suggests that the increase in reactive oxygen species detected in the HSCs is due to a deficiency in gene products required for the detoxification of reactive oxygen species. In fact, daily treatment of the FoxO-deficient animals with the antioxidant N-acetyl-L-cysteine resulted in the complete reversion of the HSC phenotype.

The two reports have much in common. Both support the idea that the effect of FoxO deficiency is highly dependent on context. Furthermore, in both cases, loss of FoxOs promoted entry into the cell cycle with different outcomes. Unrestricted proliferation of thymocytes and endothelial cells produced a tumor phenotype whereas HSCs exit from a quiescent state and terminally differentiate, eliminating the capacity for long-term self-renewal. Interestingly, the two studies report opposite effects on apoptosis in the different FoxO-deficient cell types. There was a decrease in apoptosis in FoxO-deficient endothelial cells and thymocytes, whereas an increase in apoptosis was observed in FoxO-deficient HSCs. The results described by Tothova et al. suggest that FoxOs may play a more general role in maintaining stem cell potential. FoxO3 is important in maintaining a quiescent pool of ovarian follicles. In FoxO3-deficient mice, reduced fertility in females is a result of uniform activation of follicle maturation and degeneration in the ovary. In broader terms, this ability to maintain a quiescent state is reminiscent of the role that the *C. elegans* FoxO ortholog, Daf-16, plays in entering and maintaining the dauer state of developmental arrest during which the worms develop impermeable cuticles and are nonfeeding for up to 3 months.

Although these two reports make a significant contribution to our understanding of the importance of FoxOs at the organismal level many questions remain to be explored. Why does disruption of FoxO produce such a restricted tumor phenotype? What other factors contribute to the context-dependant effects of FoxO deficiency? As FoxO proteins play a role in longevity, how might maintenance of stem cell populations and protection from stress have an impact on life span? How might these differences be exploited for the treatment of disease? Molecules exist that maintain FoxO nuclear localization, and thus maintain FoxO activity (Kau

et al., 2003; Schroeder et al., 2005), but whether any of these compounds will surmount the significant obstacles required to enter the clinic remains to be seen. The important findings reported by Paik et al. and Tothova et al. have substantially contributed to our understanding of FoxO proteins and illuminate the complexity of their function. Moreover, the generation of these animals in which FoxO inactivation can be executed in a controlled manner will allow other fundamental questions to be addressed.

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PAK-in' Up cGMP for the Move

Jeffrey Settleman[1,*]
[1]MGH Cancer Center and Harvard Medical School, 149 13th Street, Charlestown, MA 02129, USA
*Correspondence: settleman@helix.mgh.harvard.edu

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Although guanylyl cyclases have been implicated in cell migration, how they are activated to promote cell motility is unknown. In this issue, Guo et al. (2007) report direct communication between guanylyl cyclases and the Rac-p21-activated kinase (PAK) signaling pathway—which is essential for cell migration—to promote cell motility, through allosteric activation of guanylyl cyclases by autophosphorylated PAK.

Cell motility is a complex biological process that requires the precisely coordinated function of numerous cellular proteins. Fundamentally, motility requires two elements—signal transduction pathways that are activated in response to stimuli that induce motility and cellular components that mediate the mechanical aspects of motility. With regard to signaling pathways, much evidence supports an essential role for some of the Rho-family GTPases (and many of their upstream regulators and downstream effectors) as well as several receptor kinases, G protein-coupled receptors, and a variety of additional intracellular signaling proteins and scaffolds. Among the many proteins required for the mechanical aspects of cell motility are components of the actomyosin cytoskeleton, microtubules, integrins, and a variety of cytoskeletal binding proteins. Numerous studies have begun to elucidate signaling pathways that link cell surface receptors (engaged by known chemotactic ligands) and integrins to intracellular pathways that promote the coordinated cytoskeletal reorganization required for proper cell movement. In addition to the various cell-migration proteins that have been linked to specific cellular pathways, there are additional proteins that appear to be required for motility but whose specific function has been elusive. A good example of such a class of proteins is the family of guanylyl cyclases, which catalyze the conversion of GTP to the second messenger cyclic GMP (cGMP). In particular, the transmembrane subclass of guanylyl cyclases—whose activity can be modulated by intracellular signals via membrane-associated receptors—has been implicated in chemotactic cell migration and axon guidance (Ayoob et al., 2004; Bosgraaf et al., 2002). Moreover, cGMP has also been implicated in cell motility, along with a few cGMP-dependent kinases and phosphodiesterases (Postma et al., 2004). However, a signaling pathway wherein guanylyl cyclases are activated in the context of cell migration has not been established. In this issue, Guo et al. (2007) reveal a signaling link between the Rac GTPase and the transmembrane guanylyl cyclases that mediates growth factor-induced migration of fibroblasts.